Chapter 5
Transfer to Wheat of Potentially New Stem Rust Resistance Genes from *Aegilops speltoides*

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Abstract Stem rust resistance genes have been found in four different sources of *Aegilops speltoides*. These include diploid accessions AEG357-4 and AEG874-60 and the amphiploids Chinese Spring/*Ae. speltoides* TA8026 and TS01. Stem rust resistance was mapped to the 2S chromosomes derived from each of these lines. The previously reported 2B-2S#3 translocation derived from AEG357-4 was found to carry two stem rust resistance genes, here temporarily named *SrAes2t* and *SrAes3t*. The resistance genes found on the 2S chromosomes each derived from TA8026, TS01 and AEG874-60 are named *SrAes4t*, *SrAes5t* and *SrAes6t*, respectively. Lines carrying genes *SrAes2t* and *SrAes3t* are being distributed to wheat breeding programs around the world.

Keywords *Aegilops speltoides* • Resistance genes • Stem rust

Introduction

The fungal disease stem rust, caused by *Puccinia graminis* f. sp. *tritici*, has long been a scourge of wheat crops since the beginnings of agriculture. Uredia have even been found on ancient wheat spikelets estimated at 3,300 years old (Kislev 1982).

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Recently, the appearance and spread of mutants of the Ug99 stem rust lineage (summarized in Mago et al. 2013) has raised the awareness of the need to search for new resistance genes for use in wheat cultivars. Most of the currently named stem rust genes in wheat originate from related species (e.g., *Secale cereale*, *Triticum monococcum*, *T. turgidum*, *T. timopheevii*, *Thinopyrum ponticum*, *Aegilops speltoides*, *Ae. tauschii* and *Ae. ventricosum*). Many of these genes (e.g., *Sr2*, *Sr22, Sr24*, *Sr26*, *Sr36* and *Sr38*) are widely used in agriculture.

The diploid species *Ae. speltoides* Tausch. (2n = 2x = 14) has provided several resistance genes against stem rust, namely *Sr32*, *Sr39*, *Sr47*, *SrAes1t* and *SrAes7t* (summarized in Mago et al. 2013), and leaf rust (caused by *P. triticina*), namely *Lr28*, *Lr35*, *Lr36*, *Lr47*, *Lr51* and *Lr66* (summarized in McIntosh et al. 2013). To date, none of these genes are deployed in agriculture. This work describes the discovery of potentially new stem rust resistance genes in four different sources of *Ae. speltoides*.

Materials and Methods

Diploid accessions of *Aegilops speltoides* AEG357-4 and AEG874-60 were provided courtesy of the The Harold and Adele Lieberman Germplasm Bank, Tel Aviv University, Israel. Amphidiploids of Chinese Spring/*Ae. speltoides* TS01 and TA8026 were provided courtesy of the Weizmann Institute, Rehovot, Israel and Wheat Genetics Resource Center, Kansas State University, USA, respectively.

Diploid *Ae. speltoides* accessions were crossed with cultivar Angas (from Dr Hugh Wallwork, Urrbrae, Australia) using wheat as the female parent. F_1 seedlings were treated with 0.07 % colchicine (described in Dundas et al. 2008). Hybrids were crossed as females with Angas and later with cv. Westonia up to BC_5. The amphiploid accessions were crossed with Angas then backcrossed with Westonia as the female parent to BC_5.

Identification of backcrossed plants carrying each of the *Ae. speltoides* S-genome chromosomes using RFLP markers has been described in Dundas et al. (2008). PCR markers for 2S chromosomes were obtained from Mago et al. (2009) (Sr39#22r) and Seyfarth et al. (1999) (35R2/ BCD260F1). Backcrossed lines carrying S-genome chromosomes were tested for stem rust resistance at the University of Sydney (*Pgt* 34-1,2,3,4,5,6,7) and at Urrbrae (*Pgt* 343-1,2,3,5,6). Selected lines were screened against Ug99 races TTKSK, TTKST (+*Sr24*) and TTTSK (+*Sr36*) at the USDA Cereal Disease Laboratory, St. Paul, USA. Genomic in situ hybridization (GISH) was conducted on mitotic chromosome spreads using the procedure described in Mago et al. (2013).

Results and Discussion

Ae. speltoides **AEG357-4** Lines carrying the T2BS-2S#3S.2S#3L-2BL recombinant chromosome derived from AEG357-4 (Dundas et al. 2008) showed the loss of distal *Ae. speltoides* 2S#3 chromosome segments and the retention of proximal 2S#3 chromosome segments (Figs. 5.1a and 5.2). This line was found to be resistant to stem rust pathotypes *Pgt* 34-1,2,3,4,5,6,7, *Pgt* 343-1,2,3,5,6 (Dundas et al. 2008) and TTKST (pers. comm., Professor Z. Pretorius, University of Free State, Bloemfontein, South Africa). After crossing this recombinant line with Sears' *ph1b* mutant, we selected five lines derived from a F_3 population of 155 seedlings showing confirmed dissociation of RFLP and PCR markers specific for the 2S#3 chromosome (Fig. 5.2). All lines showed resistance to Australian stem rust pathotypes and Ug99 races TTKSK, TTKST and TTTSK (Table 5.1). The dissociation plants #3 and #20 showed only 2S#3S markers indicating that a stem rust resistance gene locates on the short arm of the 2S#3 chromosome (Fig. 5.2). Dissociation plant #27

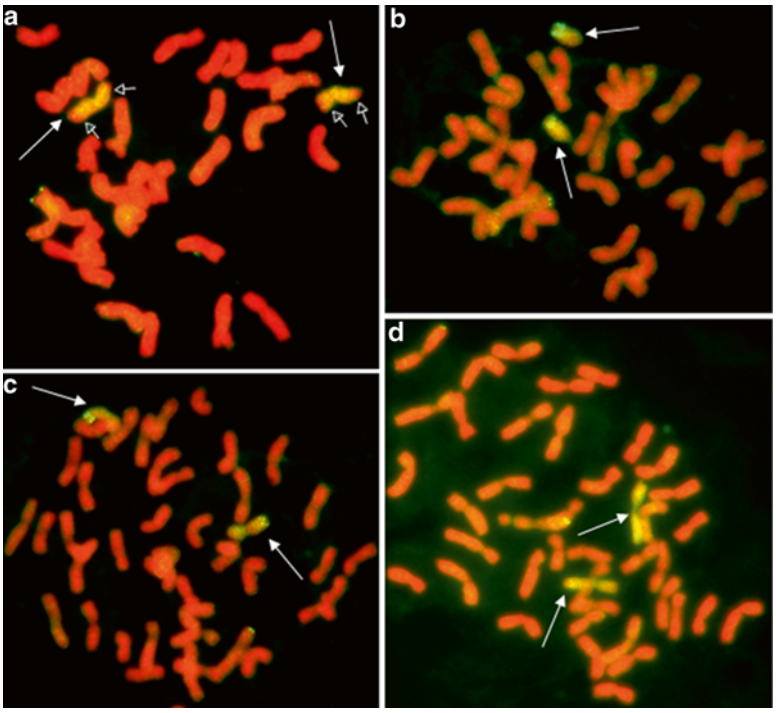

Fig. 5.1 Genomic in situ hybridization using *Aegilops speltoides* genomic DNA as probe on (**a**) T2BS-2S#3S.2S#3L-2BL translocation line, (**b**) 2S#4L ditelocentric addition line, (**c**) 2S#5 disomic addition line and (**d**) 2S#6 disomic addition line. *Solid arrows* show *Ae. speltoides* 2S chromosome segments. *Open arrows* show translocation breakpoints

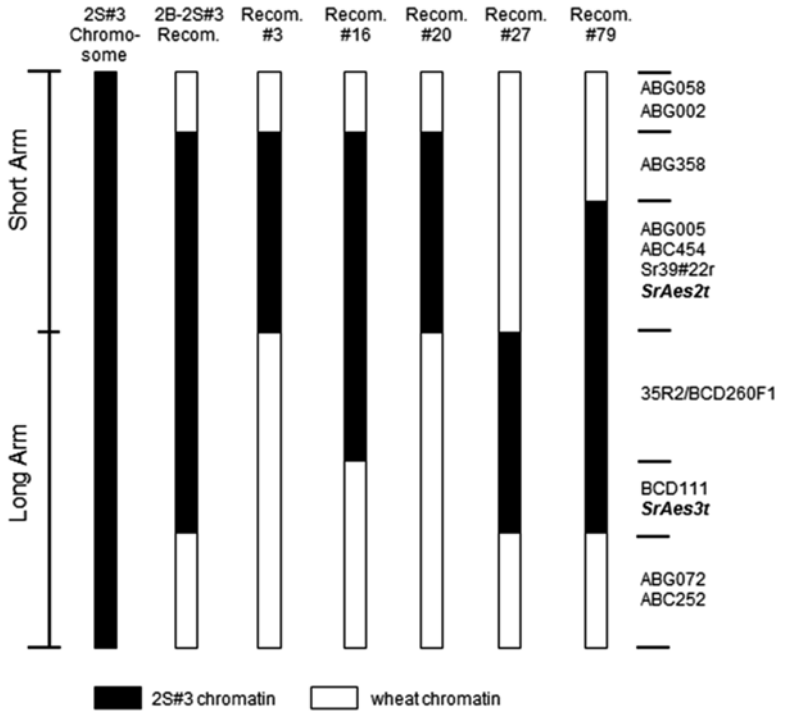

Fig. 5.2 Diagrammatic representation of wheat-chromosome 2S#3 recombinant lines showing the positions of RFLP and PCR markers and stem rust resistance genes. *Aegilops speltoides* and wheat chromatin are represented in *black* and *white*, respectively. The primary recombinant 2B-2S#3 and secondary recombinants #3, #16, #20, #27 and #79 are resistant to stem rust pathotype tested

Table 5.1 Stem rust infection types (ITs) of wheat lines carrying *Aegilops speltoides* 2S chromosomes to Ug99 pathotypes TTKSK, TTKST and TTTSK, and two Australian pathotypes

	Stem rust pathotype				
Line	TTKSK (Ug99)	TTKST (Ug99 + *Sr24*)	TTTSK (Ug99 + *Sr36*)	343-1,2, 3,5,6	34-1,2,3,4, 5,6,7
Westonia + T2B-2S#3				;	2-
Westonia + 2S#3 recomb #3	2+	22+	2	;	
Westonia + 2S#3 recomb #16	;2--	;2-	;2-	;	
Westonia + 2S#3 recomb #20	;2-	-	-	;	
Westonia + 2S#3 recomb #27	2	2-	2-	;	
Westonia + 2S#3 recomb #79	2	;2-	;2-	;	
Westonia + 2S#4 addition	;/;N	;1	;1	;	
Westonia + 2S#5 addition	;1	;/2-	;/;2-	;	
Westonia + 2S#6 addition				;	2-
Westonia	4	4	4	4	
Angas	3	3+	3+		

The 2S#3, 2S#4, 2S#5 and 2S#6 chromosomes were derived from diploid line AEG357-4, CS/*Ae. speltoides* amphiploid TA8026, CS/*Ae. speltoides* amphiploid TS01 and diploid line AEG874-60, respectively. An IT of '3' or '4' is susceptible

showed only 2S#3L markers suggesting that a stem rust resistance gene is located on the long arm of the 2S#3 chromosome (Fig. 5.2).
GISH studies on the five stem rust resistant secondary recombinants failed to reveal 2S#3 chromatin, which suggested that very small segments of 2S#3 chromatin were present on these secondary recombinants. We temporarily name the resistance gene on the short arm of the 2S#3 chromosome as *SrAes2t* and the gene on the long arm as *SrAes3t*.

Chinese Spring/*Ae. speltoides* **Amphiploid TA8026** Stem rust resistant backcrossed lines derived from TA8026 were isolated which carried only the group 2 long arm RFLP markers BCD111, ABG072 and ABC252 specific for the 2S#4 chromosome. Group 2 short arm probes ABG058, BCD221 and ABC454 did not detect the presence of 2S chromatin. GISH analysis of these lines showed the presence of either a wheat-2S#4L translocation or 2S#4L telocentric chromosome (Fig. 5.1b). The line carrying the telocentric 2S#4L chromosome was resistant to Australian and Ug99 stem rust races (Table 5.1). The stem rust resistance gene on the 2S#4L chromosome has been temporarily named *SrAes4t*.

Chinese Spring/*Ae. speltoides* **Amphiploid TS01** Backcrossed lines derived from the amphiploid TS01 were isolated which carried group 2 RFLP markers for the probes ABG002, ABC358 and ABC454, the PCR marker Sr39#22r (short arm), and RFLP markers for probes BCD111, ABG072 and PCR marker 35R2/ BCD260F1 (long arm), specific for the 2S#5 chromosome. Rust tests showed these lines to be resistant to Australian stem rust and Ug99 pathotypes (Table 5.1). GISH analysis of the line showed the presence of an apparently entire *Ae. speltoides* 2S#5 chromosome (Fig. 5.1c). The stem rust resistance gene on that chromosome is here named as *SrAes5t*.

Ae. speltoides **AEG874-60** Dundas et al. (2008) reported that the diploid accession AEG874-60 was resistant to stem rust races *Pgt* 34-2,12,13, *Pgt* 34-1,2,3,4,5,6,7 and *Pgt* 98-1,2,3,5,6. BC_5 plants with cv. Westonia were isolated carrying only the 2S#6 chromosome from AEG874-60 and carried *Ae. speltoides*-specific markers ABG058, BCD221, ABG002, ABG358, ABC454 and Sr39#22r (short arm) and BCD111, ABG072, ABC252 and 35R2/BCD260F1 (long arm). Plants with the 2S#6 chromosome were resistant to stem rust *Pgt* 34-1,2,3,4,5,6,7 and *Pgt* 343-1,2,3,5,6 (Table 5.1). GISH analysis showed an entire 2S#6 chromosome present (Fig. 5.1d). The stem rust resistance gene on that chromosome is here named as *SrAes6t*.

It is unknown if the stem rust resistance genes described herein are different from those previously reported on *Ae. speltoides* 2S chromosomes (namely *Sr32*, *SrAes1t*, *SrAes7t*, *Sr39* and *Sr47*). Lines carrying the T2BS-2S#3S.2S#3L-2BL chromosome with genes *SrAes2t* and *SrAes3t* are being distributed to many wheat breeding programs around the World.

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